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TECHNICAL MANUSCRIPT 561

THE AGGLUTININ RESPONSE OF RABBITS TO COMBINED PASTEURELLA TULARENSIS AND BRUCELLA ABORTUS VACCINATION

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OCTOBER 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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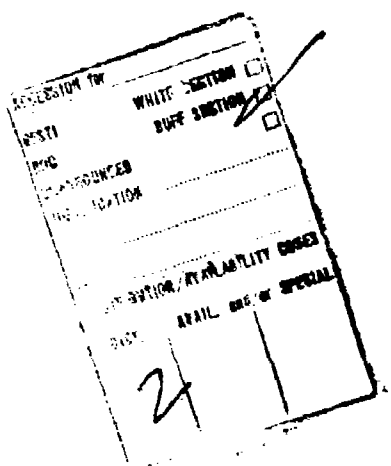

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TECHNICAL MANUSCRIPT 561

THE AGGLUTININ RESPONSE OF RABBITS TO COMBINED PASTEURELLA TULARENSIS AND BRUCELLA ABORTUS VACCINATION

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BIOLOGICAL SCIENCES LABORATORIES

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In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ABSTRACT

A schedule was developed for the simultaneous production of a Pasteurella tularensis and Brucella abortus antiserum in rabbits. Three doses of 10^8 viable P. tularensis LVS organisms were given intravenously at weekly intervals. One day prior to the final dose of P. tularensis, the rabbits received 10^9 viable cells of B. abortus strain 19 intravenously. The use of live vaccines, administered in this sequence, resulted in high agglutinin titers within 3 weeks. The maximal agglutinin titer to either organism was observed 1 week after the final injection.

I. INTRODUCTION*

A single antiserum capable of reacting with more than one bacterium would facilitate serological testing and studies. A procedure was developed for the production of anti-Pasteurella tularensis antisera in rabbits, employing the attenuated live vaccine strain (LVS).[1] The purpose of the present study was to determine the feasibility of producing a bivalent antiserum by combined vaccination of rabbits with viable Brucella abortus strain 19 and P. tularensis LVS.

II. MATERIALS AND METHODS

A. ANIMALS

New Zealand white rabbits weighing between 1.8 and 2.5 kg were used. Except where noted, all experimental groups contained five animals.

B. VACCINES

The production and administration of viable tularemia vaccine have been reported.[1] Desiccated Brucella abortus strain 19 vaccine was obtained from the Haver-Lockhart Laboratories, Kansas City, Mo., and reconstituted according to the manufacturer's directions. The reconstituted vaccine was diluted in saline to obtain the desired concentrations. The number of viable bacteria in either vaccine was estimated by plating appropriate dilutions of the vaccines on glucose-cysteine-blood agar.[2]

C. AGGLUTINATION TECHNIQUES

Anti-P. tularensis agglutinin titers were determined using a formalinized suspension of the virulent strain SCHU organisms.[3] Brucella tube agglutinating antigen was obtained from the U.S. Department of Agriculture and the same technique was used to assess Brucella agglutinins.

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III. RESULTS

A. INTRAVENOUS VACCINATION WITH VIABLE B. ABORTUS STRAIN 19

The B. abortus agglutinin titers of sera from three groups of rabbits administered various intravenous (IV) doses of viable B. abortus strain 19 organisms are presented in Table 1. Three of five rabbits survived the initial administration of 10^{10} organisms but only one of these survived revaccination at this dose; all rabbits survived the procedure when doses of 10^9 or 10^8 organisms were used.

TABLE 1. EFFECT OF INTRAVENOUS DOSE OF BRUCELLA ABORTUS STRAIN 19 VACCINE ON AGGLUTININ PRODUCTION IN RABBITS

	Mean Reciprocal Agglutinin Titer After Indicated Dose of B. abortus Strain 19 Organisms[a]		
Day	1×10^{10}	1×10^9	1×10^8
7	2,560	2,560	1,280
14	5,120	1,280	640
21	2,560	1,280	640
28[b]	2,560	640	320
35	5,120	1,280	1,280
42	2,560	1,280	1,280
49	2,560	1,280	640
56	2,560	640	320

a. Pooled serum samples.
b. All animals revaccinated.

The highest primary and secondary response titers (1:5,120) were observed with vaccine doses of 10^{10} organisms but lethality at this concentration precluded its use for routine vaccination. Vaccination with 10^9 cells elicited an agglutinin response of 1:2,560 within 7 days after primary administration; at this time animals administered 10^8 organisms had a titer of 1:1,280. Both of the latter vaccine doses induced peak secondary response titers of 1:1,280, but the maximum secondary response was of slightly greater duration in the group that received 10^9 organisms.

B. HYPERIMMUNIZATION WITH VIABLE B. ABORTUS STRAIN 19

The agglutinin response was determined for rabbits vaccinated with eight IV doses of 10^9 viable B. abortus strain 19 organisms at weekly intervals. The maximal B. abortus agglutinin titer (1:2,560) was observed on the 14th day; all subsequent weekly titers were 1:1,280.

C. SUBCUTANEOUS ADMINISTRATION OF *B. ABORTUS* VACCINE

Following the subcutaneous (SC) administration of 10^9 viable organisms of the *B. abortus* vaccine to rabbits, the maximal agglutinin titers were fourfold lower than those observed in rabbits that received the same vaccine by the IV route. Revaccination of the animals on the 28th day did not result in an anamnestic response.

D. SIMULTANEOUS COMBINED *P. TULARENSIS* AND *B. ABORTUS* VACCINATION

A group of rabbits was vaccinated IV with both 10^9 viable *P. tularensis* LVS and 10^9 viable *B. abortus* 19 organisms. Control groups received 10^9 organisms of either the *P. tularensis* or the *B. abortus* vaccine. All animals were revaccinated with the respective vaccines on the 28th day. Following revaccination, four of the five animals that received the combined vaccine and one of five that received LVS died; deaths occurred within 48 hours.

The *P. tularensis* agglutinin titers of animals that received the combined vaccine were not markedly different from those of the animals that were administered the *P. tularensis* vaccine alone (Table 2). The animals vaccinated with *B. abortus* alone had a low level of cross-reacting *P. tularensis* agglutinins on the 7th and 14th days.

TABLE 2. *PASTEURELLA TULARENSIS* AGGLUTININ TITERS OF RABBITS INOCULATED INTRAVENOUSLY WITH COMBINED *PASTEURELLA TULARENSIS* AND *BRUCELLA ABORTUS* VACCINE OR WITH ONLY ONE VACCINE

	Mean Reciprocal Agglutinin Titer		
Day	Combined Vaccine[a]	*P. tularensis*[b]	*B. abortus*[b]
7	416	768	34
14	544	480	34
21	240	160	<10
28[c]	272	160	<10
35	320	384	<10
42	320	272	<10
49	320	192	<10
56	160	160	<10

a. Approximately 10^9 cells of each bacterium.
b. Approximately 10^9 cells.
c. All animals revaccinated.

Brucella agglutinin titers are presented in Table 3. There were no appreciable differences between titers of rabbits inoculated with combined vaccine and those of animals receiving the Brucella vaccine alone. Rabbits given the P. tularensis vaccine alone had a low level of cross-reacting antibodies on the 7th day but little or none on the 14th day and thereafter.

TABLE 3. BRUCELLA ABORTUS AGGLUTININ TITERS OF RABBITS INOCULATED INTRAVENOUSLY WITH A COMBINED PASTEURELLA TULARENSIS AND BRUCELLA ABORTUS VACCINE OR WITH ONLY ONE VACCINE

	Mean Reciprocal Agglutinin Titer		
Day	Combined Vaccine[a]	P. tularensis[b]	B. abortus[b]
7	1,088	15	1,280
14	384	<10	640
21	192	<10	272
28[c]	120	<10	272
35	160	<10	416
42	320	<10	384
49	320	<10	320
56	160	<10	320

a. Approximately 10^9 cells of each bacterium.
b. Approximately 10^9 cells.
c. All animals revaccinated.

Anamnestic responses were not observed following revaccination with either vaccine alone; the one animal surviving revaccination with the combined vaccine did not show an anamnestic response to either vaccine.

In a subsequent study of the lethality of combined simultaneous vaccination of rabbits with 10^9 viable P. tularensis LVS and 10^9 viable B. abortus strain 19 organisms, death occurred in nine of 15 animals following primary vaccination and in five of six following secondary administration.

E. SEQUENTIAL ADMINISTRATION OF B. ABORTUS AND P. TULARENSIS VIABLE VACCINES

Bacterial agglutinin titers in rabbits following sequential IV administration of 10^9 viable B. abortus strain 19 and of 10^9 P. tularensis LVS (in either order after 24 hours) are presented in Table 4. When the vaccination sequence was P. tularensis LVS followed 24 hours later by B. abortus strain 19, three of the five rabbits died within 4 days after primary vaccination. Agglutinin titers of the two surviving animals were at least 1:1,280 to either organism during the primary and secondary responses.

TABLE 4. AGGLUTININ RESPONSES OF RABBITS VACCINATED BY THE IV ROUTE SEQUENTIALLY WITH *BRUCELLA ABORTUS* STRAIN 19 AND *PASTEURELLA TULARENSIS* LVS

Vaccine Sequence	Agglutination Antigen	Reciprocal Agglutinin Titer on Day Indicated[a]							
		7	14	21	28[b]	35	42	49	56
P. tularensis; after 24 hours, *B. abortus*[c]	*P. tularensis*	1,280	1,280	1,280	640	1,280	1,280	640	160
	B. abortus	2,560	2,560	1,280	1,280	1,280	2,560	2,560	1,280
B. abortus; after 24 hours, *P. tularensis*[d]	*P. tularensis*	640	640	320	320	1,280	1,280	640	320
	B. abortus	2,560	2,560	2,560	1,280	2,560	2,560	1,280	1,280

a. Pooled serum samples.
b. Animals revaccinated in same sequence.
c. Three of five rabbits died within 4 days after primary vaccination.
d. No deaths resulted from vaccination procedure.

When the vaccination sequence was B. abortus strain 19 followed 24 hours later by P. tularensis LVS, none of the animals died. During the primary response to this vaccination procedure P. tularensis but not B. abortus agglutinin titers were slightly lower than those obtained when the sequence was reversed. Secondary response anti-P. tularensis titers and both the primary and secondary response B. abortus agglutinin titers were similar to those obtained with the reverse (P. tularensis LVS - B. abortus strain 19) vaccine sequence. With the minor exception noted above, these agglutinin titers were comparable to those previously obtained when each vaccine was administered alone.

Previous studies[1] had demonstrated that short-term hyperimmunization with P. tularensis LVS increases the P. tularensis agglutinin titers of rabbits. This procedure was combined with that of sequential immunization used in the current studies. Three doses of 10^8 viable P. tularensis LVS cells were administered IV at weekly intervals to 10 rabbits; 24 hours prior to the final dose, the animals received 10^9 viable cells of B. abortus IV.

The agglutinin titers are presented in Table 5. The highest mean titer to either organism (1:1,408) was obtained 7 days after the last P. tularensis injection; titers declined to 1:320 or less 56 days after the initial inoculation with P. tularensis LVS. This procedure did not result in death of any animals and is recommended for rapid production of a bivalent P. tularensis and B. abortus antiserum in rabbits.

TABLE 5. AGGLUTININ RESPONSES OF RABBITS FOLLOWING HYPERIMMUNIZATION WITH PASTEURELLA TULARENSIS LVS AND A SINGLE ADMINISTRATION OF BRUCELLA ABORTUS STRAIN 19[a/]

Day	Mean Reciprocal Agglutinin Titer	
	P. tularensis	B. abortus
7	272	-
14	704	-
21	1,408	1,408
28	896	1,280
35	544	768
42	352	640
49	256	448
56	208	320

a. Doses of 10^8 viable LVS given IV at three weekly intervals to 10 rabbits; 24 hours prior to the final LVS dose, 10^9 viable cells of strain 19 were administered IV.

IV. DISCUSSION

The B. abortus strain 19 vaccination schedule for the production of maximal antibody levels in rabbits was similar to a regimen successfully used with virulent organisms.[4] One IV dose of 10^9 viable cells was sufficient to produce high agglutinin titers. A hyperimmunization procedure did not result in improved titers; this is in contrast to some procedures for nonviable vaccines and chemically purified antigens that require prolonged administration schedules. This example, as well as one involving P. tularensis,[1] is indicative of the subtleties one may encounter when viable attenuated organisms are used. Antibody formation is dependent on the in vivo growth and antigen production of the bacteria and probably varies with the host-parasite combination.

Mortality occurred in some experiments on combined vaccination with the two live vaccines. Pasteurella tularensis does not have classic endotoxin[5] but B. abortus does possess endotoxic activity.[6] Possibly P. tularensis administered prior to or simultaneously with B. abortus can prime the rabbit for the action of B. abortus endotoxin. It is known[1] that larger doses of viable P. tularensis LVS are toxic for the rabbit when administered by the IV route. The mortality in the present study might be attributable to potentiation of this system by B. abortus endotoxin. Regardless, the observations made point up the necessity for employing various schedules when developing a satisfactory procedure for administration of two live vaccines to produce maximal simultaneous antibody responses.

The cross reactivity between B. abortus and P. tularensis agglutinins was generally low (<1:40). Reduction of the antisera with 2-mercaptoethanol completely abolished the heterologous reactivity in sera from rabbits given a single vaccine; chemical reduction also resulted in approximately an eightfold decrease in homologous titers against both organisms on the 21st day of the combined procedure.*

A procedure for the rapid production of a bivalent antiserum with antibody levels comparable to those produced by single vaccination with each organism was established. It is a practical procedure because both live vaccines are produced from attenuated strains and can be used without extensive safety equipment; both vaccines can be easily prepared in the laboratory or purchased; the antiserum can be collected within 3 weeks after initiation of the vaccination; and there is the theoretical advantage of employing live vaccines with unaltered antigens for the production of antibodies.

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* Unpublished observations.

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13. ABSTRACT

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14. Key Words

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Agglutinin
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